Federal Democratic Republic of Ethiopia

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ET 475 (2000) (English): SEEDS - BIOCHEMICAL TEST FOR VIABILITY (TOPOGRAPHICAL TETRAZOLIUM TEST)

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ETHIOPIAN STANDARD

ES 475:2000

(Reaffirmed :2012)

First edition

Seeds - Biochemical test for viability (Topographical tetrazolium test)

ICS: 65.020.20

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Foreword

This Ethiopian Standard has been prepared under the direction of Technical Committee for seeds (TC 16) and published by the Ethiopian Standards Agency (ESA).
The technical contents in the text of this Standard are identical with ES 475:2000 edition while the reissued standard carries some editorial and technical changes observed in the course of systematic review.

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Seeds — Biochemical test for viability (Topographical tetrazolium test)

1 Scope

This Ethiopian Standard specifies the quick determination of the viability of seed samples in the case of species which normally germinate slowly or show dormancy when determined in accordance with the method of test specified in ES 474.

2 Normative references

The following standard contain provision which, through reference in this text, constitute provision of this Ethiopian Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this Ethiopian Standard are encouraged to investigate the possibility of applying the most recent editions of the Ethiopian Standards indicated below. Registers of currently valid standards are maintained in the Quality and Standards Authority of Ethiopia.

ES 471:2000, *Seeds – Sampling.*

ES 474:2000, *Seeds – Germination test.*

3 Reagents

During this test, use only reagent of recognised analytical grade and distilled water.

3.1 A 0.1% or aqueous solution of tetrazolium chloride or bromide shall be used. If the pH of the distilled water available is not within the range of 6.5-7.5, the solution shall be buffered as follows:

3.2 Two solutions shall be prepared as follows:

3.2.1 Solution 1 - dissolve 9.078g $KH_2 PO_4$ in 1000ml water.

3.2.2 Solution 2 - dissolve 9.472g $Na_2 HPO_4. 2H_2 O$ in 1000 ml water or dissolve 11.8767g $Na_2 HPO_4$ in 1000 ml water.

3.3 Mix parts of solution 1 of solution 2 to form the buffer solutions.

3.4 Dissolve the correct amount of (either chloride or bromide) solution in the proportion of 10g of the salt to 1000 ml of the buffer solution, to make 1% solution of the tetrazolium salt.

4 Field of application

The test shall not be valid for previously germinated seeds and must not be applied to submitted samples, which contain any dry germinated seeds.

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5 Principle

5.1 In a biochemical test, evidence of the reduction processes which take place in living cells is provided by the reduction of an indicator. In the topogaphical tetrazsolium test a colorless solution of 2, 3, 5- triphenyl tetrazolium chloride or bromide is used as an indicator and imbibed by the seed. Within the seed tissues it interacts with the reduction processes of living cells and accepts hydrogen from the dehydrogenase. By hydrogenation of the 2, 3, 5- triphenyl-tetrazolium chloride or bromide, a red, stable and non-diffusible substance, triphenyl formazan, is produced in living cells. This makes it possible to distinguish the red colored living parts of seeds from the colorless dead unstained non-viable seeds, partially stained seeds may occure.varying proportions of necrotic tissues are found in different zones of these partially stained seeds. The position and size of the necrotic areas in the embryo and/or endosperm game to phyte tissue, and not necessarily the intensity of colour, determine whether such seeds are classified as viable or nonviable, not necessarily the intensity of colour. However, colour differences along with tissue soundness are to be considered decisive mainly to the extent that they permit recognition and location of sound, weak or dead tissue.

6 Sampling

Samples shall be taken in accordance with ES 471.

7 Procedure

7.1 Working sample

The test shall be carried out on four replicates of 100 seeds drawn at random from the pure seed fraction of a purity test or an individual seed that are found dormant at the end of germination tests.

7.2 Determination

7.2.1 The seed shall be prepared in order to ficilitate penetration of the tetrazolium solution. the prepared seeds or embryos are then completely immersed in the tetrazolium solution and maintained at a temperature of 30^0C in complete darkness for a period dependent on the species. At the end of this period the solution is decanted. After rinsing with water, the treated seeds or embryos shall be spread on a plate and kept wet for examinations.

7.2.2 Each seed shall be evaluated as viable or nonviable on the basis of the staining pattern revealed in this examination.

8 Expression of results

In testing a sample, the number of seeds considered viable is determined in each of the four replicates. Maximum tolerated ranges for replicate differences are the same as for germination tests therein. The average percentage shall be calculated to the nearest whole number and reported.

9 Test report

The test report shall include the following information:

9.1 Tetrazolium test,% of seeds are viable.

9.2 In the case of species of the Leguminosae, the following shall be reported additionally.

Percentage of hard seeds found in the test and percentage of viable seeds.

9.3 Further details shall be given at the discretion of the testing station e.g. percentage empty, with larvae, broken or decayed seeds.

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9.4 When individual seeds shall be tested at the end of the germination test, the result shall be reported in accordance with ES 474.

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Ethiopian Standards Agency

Organization and Objectives

The Ethiopian Standards Agency (ESA) is the national standards body of Ethiopia established in 2010 based on regulation No. 193/2010.ESA is established due to the restructuring of Quality and Standards Authority of Ethiopia (QSAE) which was established in 1970.

ESA's objectives are:-

- Develop Ethiopian standards and establish a system that enable to check weather goods and services are in compliance with the required standards,
- Facilitate the country's technology transfer through the use of standards,
- Develop national standards for local products and services so as to make them competitive in the international market.

Ethiopian Standards

The Ethiopian Standards are developed by national technical committees which are composed of different stakeholders consisting of educational Institutions, research institutes, government organizations, certification, inspection, and testing organizations, regulatory bodies, consumer association etc. The requirements and/ or recommendations contained in Ethiopian Standards are consensus based that reflects the interest of the TC representatives and also of comments received from the public and other sources. Ethiopian Standards are approved by the National Standardization Council and are kept under continuous review after publication and updated regularly to take account of latest scientific and technological changes. Orders for all Ethiopian Standards, International Standard and ASTM standards, including electronic versions, should be addressed to the Documentation and Publication Team at the Head office and Branch (Liaisons) offices. A catalogue of Ethiopian Standards is also available freely and can be accessed in from our website.

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International Involvement

ESA, representing Ethiopia, is a member of the International Organization for Standardization (ISO), and Codex Alimentarius Commission (CODEX). It also maintains close working relations with the international Electro-technical Commission (IEC) and American Society for Testing and Materials (ASTM).It is a founding member of the African Regional Organization for standardization (ARSO).

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